Table 1. Incidence of Leukæmia in Three Strains of Mice receiving Cell-free ('Selas' Filtered) Extracts of Leukæmic *AKR* Mouse Tissues in the Newborn Period

Strain		Total animals	Leukæmia	Mean age of leukæmia development
C3Hf/Gs	Injected	36	10 (27·8%)	8·7 months
	Controls	83	0	—
C57BR/cd	Injected	47	7 (14·9%)	10·3 months
	Controls	120	4 (3·3%)	18·5 months
C3Hf × *AKR* F_1 Hybrid	Injected	51	11 (21·6%)	9·5 months
	Controls	65	5 (7·7%)	9·4 months

at *p*H 7·5 at 25° for 18 hr. After removal of sodium dodecyl sulphate at 0° as the insoluble potassium salt, the extract was deproteinized in the cold at *p*H 4·3 in the presence of 10 per cent sodium xylene sulphonate. After neutralization, the nucleic acids were precipitated by the addition of an equal volume of 98 per cent isopropyl alcohol. The isolated nucleic acids were dissolved in a minimum amount of water and sodium xylene sulphonate was added to a concentration of 30 per cent at *p*H 7·5 (to dissociate any residual nucleoprotein). After the addition of two volumes of water, the deproteinization was repeated at *p*H 4·3 and 0°. The nucleic acids were re-precipitated with isopropyl alcohol, isolated, redissolved and fractionally precipitated with 0·7 vol. isopropyl alcohol in the presence of 0·3 *M* sodium acetate and 0·01 *M* versene. The resulting precipitate of deoxyribonucleic acid and ribonucleic acid (in almost quantitative yield) was finally washed with ethyl alcohol and dissolved to a concentration of 0·1 per cent in sterile 0·85 per cent sodium chloride and stored frozen until used. 0·1 ml. of this material was injected into newborn mice.

As shown in Table 2, seventeen *C3Hf/Gs* and forty-one *C57BR/cd* animals were injected with the nucleic acid preparations from leukæmic *AKR* tissues. No leukæmias developed in these animals. Of the twenty-three *C3Hf* × *AKR* F_1 hybrid animals injected with non-leukæmic *AKR* deoxyribonucleic acid – ribonucleic acid, six developed leukæmia. Five of eight animals receiving deoxyribonucleic acid – ribonucleic acid from leukæmic *AKR* organs developed leukæmia. Five spontaneous leukæmias occurred in two litters out of fourteen non-injected control litters (sixty-five animals). All these results are statistically significant at 5 per cent level with the χ^2 test.

Table 2. Incidence of Leukæmia in Three Strains of Mice receiving Nucleic Acid Preparations (DNA–RNA) of *AKR* Mouse Tissues in the Newborn Period

Strain		Total animals	Leukæmia	Mean age of leukæmia development
C3Hf/Gs	Injected with leukæmic *AKR* DNA–RNA	17	0	—
	Controls	83	0	—
C57BR/cd	Injected with leukæmic *AKR* DNA–RNA	41	0	—
	Controls	120	4 (3·3%)	18·5 months
C3Hf × *AKR* F_1 hybrid	Injected with non-leukæmic *AKR* DNA–RNA	23 } 31	6 } 11 (35·4%)	11·4 months
	Injected with leukæmic *AKR* DNA–RNA	8	5	11·3 months
	Controls	65	5 (7·7%)	9·4 months

It is emphasized that the deoxyribonucleic acid – ribonucleic acid from the non-leukæmic *AKR* mice, as well as that from the leukæmic animals of the same strain, was apparently active in producing an incidence of leukæmia in the hybrids comparable to that of cell-free extract injected animals.

Two of the leukæmias developing in the deoxyribonucleic acid – ribonucleic acid-injected group and one in the non-leukæmic deoxyribonucleic acid – ribonucleic acid group were transferred by cells to F_1 hybrid animals and to animals of the two parent strains. The tumour grew progressively, resulting in death of the hybrid recipients, but did not grow in *AKR* or *C3H* animals.

These preliminary results in hybrid animals suggest that free nucleic acid is leukæmogenic, although the possibility is not ruled out that a virus or provirus is, in fact, the active agent in cell-free extracts, and that our preparations contain active virus nucleic acid. It is unlikely, however, that intact active virus could survive the nucleic acid isolation procedure. Experiments are now in progress to test the activity of deoxyribonucleic acid – ribonucleic acid obtained from the tissues of low-leukæmia mice and from other species and to determine the effect on active cell-free extracts of deoxyribonuclease and ribonuclease. A full report of these investigations will be published separately.

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Sensitization by Œstradiol to the Production of Experimental Nephrocalcinosis

Earlier experiments showed that excessive oral administration of mono- or dibasic-phosphates produces nephrocalcinosis in the rat, and that these renal lesions are aggravated by mineralocorticoids, such as deoxycorticosterone[1–3]. Recent experiments revealed that, under similar experimental conditions, the nephrocalcinosis produced by oral administration of acid sodium phosphate is not aggravated by methyltestosterone, progesterone, corticosterone, Δ^1-cortisol acetate, or Δ^1-cortisone acetate, but curiously, œstradiol proved to be even more effective in this respect than deoxycorticosterone. It has long been known that folliculoid or 'œstrogenic' hormones exert a pronounced effect on calcium metabolism in birds, presumably because in the avian species the production of calcified egg shells is closely related to the action of sex hormones. In mammals, on the other hand, folliculoids do not exert any comparably pronounced effect upon calcium

Table 1

Group	Œstradiol	NaH_2PO_4 (per cent)	Nephrocalcinosis (Scale 0–3)	Mortality (per cent)	Duration of experiment (days)
I	none	10	0·6	0	12
II	500 μgm.	10	2·2	40	12
III	none	10	0·2	0	15
IV	500 μgm.	10	2·3	10	15
V	none	20	0·2	0	5
VI	500 μgm.	20	*	100	5
VII	none	30	1·3	0	9
VIII	500 μgm.	30	3·0	80	9

* All animals in this group died within first three days, hence there was insufficient time for the development of marked nephrocalcinosis; besides, the intensity of calcification could not be compared with that of the corresponding controls (Group V), since the latter were killed on the fifth day.

metabolism, and, therefore, the production of intense nephrocalcinosis by œstradiol (when given in combination with phosphates) was made the subject of a special study.

Eighty female Sprague–Dawley rats with an average body-weight of 100 gm. (range: 95–111 gm.) were subdivided into eight equal groups and treated as indicated in Table 1.

Œstradiol was administered in the form of microcrystals at the daily dose of 500 μgm. in 0·2 ml. of water, subcutaneously. Aqueous solutions of acid sodium phosphate were given, by stomach tube, in two daily doses of 2 ml.

The degree of nephrocalcinosis was estimated both macroscopically (judged by the appearance of whitish spots on the outer and cut surface of the kidneys) and microscopically (after staining neutral-formalin-fixed, paraffin-embedded sections with Kossa's silver nitrate technique). The intensity of the calcification was gauged in terms of an arbitrary scale of 0–3, the means of the readings in each group being listed in Table 1.

It is evident from Table 1 that, under our experimental conditions, the 10 per cent acid sodium phosphate solution in itself caused only traces of nephrocalcinosis, but resulted in marked renal calcification when given conjointly with œstradiol. Otherwise untreated rats tolerated even 20 or 30 per cent acid sodium phosphate under these conditions, while in animals simultaneously treated with œstradiol, these concentrated phosphate solutions resulted in a high mortality and an aggravation of the nephrocalcinosis.

Since œstradiol is completely devoid of mineralocorticoid, and deoxycorticosterone of folliculoid actions, the enhancement of nephrocalcinosis appears to be an 'independent steroid hormone action', in the sense in which this term has been defined[4].

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Fluoride Removal by Powdered Dental Enamel from Solutions of Stannous or of Sodium Fluoride

In 1955, Hatton, Nebergall and Muhler[1] described an experiment in which powdered enamel samples were shaken in dilute solutions of stannous fluoride or of sodium fluoride that initially contained equivalent concentrations of fluoride. Much more fluoride was removed (presumably taken up by the enamel) from the stannous fluoride solution; for example, when the final concentrations of fluoride were 17 μgm./ml., 7–8 times as much fluoride was removed from the stannous as from the sodium fluoride solution. Hatton *et al.* suggested that the greater initial acidity of the stannous fluoride solutions may have been in part responsible[2]. Another important *p*H effect was overlooked by Hatton *et al.*, namely, shaking with powdered enamel increased the *p*H of the solutions, markedly for stannous fluoride and detectably for sodium fluoride. Stannous fluoride solutions are not stable when the *p*H increases and even at acid *p*H's on standing. Nebergall, Muhler and Day[3] described the opalescence, "probably stannous hydroxide" (contained less than 0·01 per cent fluorine), which developed and was ultimately precipitated from a solution of *p*H 2·38. We found that by increasing the *p*H of stannous fluoride solutions in the absence of enamel, precipitates were formed which contained larger percentages of fluoride ($\geqslant$ 0·01 per cent fluoride) that might account at least in part for the greater amount of fluoride removed from stannous fluoride solutions. Amorphous precipitates were seen microscopically along with the centrifuged enamel particles when powdered enamel was shaken with stannous fluoride solutions.

Solutions of stannous fluoride (initial *p*H, 3·5) were prepared according to Nebergall[3] containing approximately 40 p.p.m. fluorine. The *p*H was adjusted using a dilute sodium hydroxide solution to approximately 4·5; the solution was protected from air and shaken mechanically for 24 hr. Following centrifugation an aliquot of the supernatant solution was taken for fluoride analysis. The precipitate was resuspended, the *p*H adjusted to approximately 5·5 and the mixture stirred for another day. This process with increments of *p*H was repeated daily for eight days; the *p*H on the final day was brought to 8·6 before the final 24-hr. agitation. Fluoride analyses of the supernatant solution showed that as the *p*H

Table 1. Overall Amounts of Fluorine (μgm.) in 50 ml. of Stannous or Sodium Fluoride Solutions Before and After Shaking with 1 gm. of Enamel. Estimates of Fluorine removed from Solution by Enamel and by Precipitation

Initial fluorine (*a*)	Stannous fluoride solution: Hatton *et al.* (*b*)	Stannous fluoride solution: Present series (*c*)	Stannous fluoride solution: Estimate of fluorine removed by enamel only (*b*–*c*)	Sodium fluoride solution. Fluorine removed by enamel (*d*)
100	50	0	50	52
250	150	135	15	65
500	300	175	125	145
750	475	275	200	175
1,000	600	375	225	225
1,250	750	475	275	225
1,500	900	575	325	250
1,750	1,050	700	350	250
2,000	1,200	750	450	250

a, Interpolated values, see text; *b*, fluorine removed from solution shaken with enamel (Hatton *et al.*, ref. 1); *c*, fluorine removed from solution by alkalinization to *p*H 6·5; *d*, Hatton *et al.* (ref. 1).